

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Serial No. : 10/519,704  
Filed : December 5, 2005  
Applicant : Christensen et al.  
Title : **Process for Making De-Esterified Pectins Their Composition and Uses Thereof**

TC/AU : 1794  
Examiner : Kelly Jo Bekker

Docket No. : 04-501 (29776-0005)  
Customer No. : **62488**

**DECLARATION UNDER 37 C.F.R. § 1.132**

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**Via EFS Web**  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

I, Jens E. Trudso, hereby declare that:

1. I am a Research Fellow at C.P. Kelco Aps, owned by J.M. Huber, and an inventor of the above-referenced patent application. I have a M.Sc.chem.eng. (degree) from the Technical University of Denmark. I have 35 years of experience in the development and application of specialty hydrocolloids.
2. I have reviewed the Office Action mailed October 22, 2009, concerning the present patent application. I also have reviewed the prior art references relied upon by the Examiner in the Office Action, including PCT Publication No. 99/37685 to Marr et al. ("Marr") and PCT Publication No. 98/58968 to Larsen et al. ("Larsen").



3. The present application relates to amidated pectins having a low degree of esterification. The amidated pectins are prepared by first using biocatalytic (e.g., enzymatic) de-esterification to reduce the degree of esterification of the pectin to between 60% and 30% without the occurrence of any significant depolymerization or loss of molecular weight. The de-esterified pectins are subsequently amidated to provide amidated pectins that have a surprisingly low tendency towards aggregation, a surprisingly high molecular weight, and a surprisingly low loss of intrinsic viscosity as compared to amidated pectins prepared using conventional methods.

4. Marr discloses that pectins having a low degree of esterification (e.g., below approximately 10) that are heat stable have problems with solubility. (Page 2, Lines 8-14). To address these problems, Marr teaches that it is desirable to reduce both the degree of esterification and the molecular weight of the pectin, either of which may be performed enzymatically *in any particular order*. (Page 2, Lines 16-24; Page 4, Lines 1-30; Page 5, Lines 7-11). The resulting pectins are described as having a “low molecular weight” (e.g., 20,000 to 50,000 Daltons) and a degree of esterification of less than approximately 20. (Page 3, Lines 1-11). These “low molecular weight pectins” optionally may be combined with a “high molecular weight pectin” (e.g., 50,000 to 150,000 Daltons) for use in various applications. (Page 3, Lines 1-7; Page 5, Lines 21-25).

5. Larsen discloses that bulk-extracted pectins produce products having undesirable properties (e.g., haze in gels, enhanced viscosity, incomplete solubility, and syneresis). (Page 4, Lines 28-34). To address these problems, Larsen teaches that it is desirable to obtain selected fractions of high-esterified pectin by consecutive extraction with acidic aqueous solutions. (Abstract). Larsen further teaches that these high-esterified pectin fractions are useful in



preparing de-esterified and amidated pectin fractions having improved functional characteristics.  
(Abstract).

6. One skilled in the art would not combine the cited references in the manner proposed by the Examiner. Marr is directed to bulk-extracted pectins while Larsen is directed to pectin fractions and teaches that use of bulk-extracted pectins, such as those disclosed by Marr, is undesirable. One skilled in the art would not rely only on Larsen's disclosure of amidation to modify Marr, while disregarding Larsen's disclosure that bulk-extracted pectins have undesirable properties.

7. Even if one skilled in the art were to combine Marr and Larsen, a more plausible combination of the references would combine the method for preparing the high-ester pectin fractions of Larsen, the de-esterification and molecular weight reduction of Marr, and the amidation of Larsen. Thus, the combined references would result in an amidated de-esterified pectin fraction having a low degree of esterification and a low molecular weight – not an amidated de-esterified pectin having a low degree of esterification and a high molecular weight.

8. Neither Marr nor Larsen disclose that it is desirable to minimize the loss of the molecular weight and the loss of the intrinsic viscosity of the pectin during its de-esterification and amidation. In fact, both Marr and Larsen teach the opposite.

- a. Marr, as noted above, expressly teaches that both the molecular weight and degree of esterification should be reduced. Those skilled in the art would appreciate that reducing the molecular weight of the pectin during de-esterification also would suggest that there was a corresponding reduction to the intrinsic viscosity of the pectin.



- b. Larsen, although not expressly teaching away from minimizing the loss of the molecular weight and the loss of the intrinsic viscosity during de-esterification and amidation, discloses in its examples that the amidated pectin fractions have a significantly decreased viscosity grade, which also would suggest that there was a corresponding reduction to the molecular weight and the intrinsic viscosity of the de-esterified and amidated pectins. (See e.g., Examples 6 and 7 (reporting a Visc° of 74 of the first pectin fraction before de-esterification and a Visc° of 21.7 after de-esterification)).

9. The amidated pectins of the claimed invention have surprisingly improved properties as compared to the amidated pectins of the prior art. By first de-esterifying the pectin with the described biocatalytic de-esterification method to a de-esterified pectin having a degree of esterification from about 60% to about 30% and subsequently de-esterifying/amidating the de-esterified pectin the depolymerization and aggregation of the deesterified pectins is significantly reduced. (Paragraph [0057]). The amidated pectins also exhibit other improved properties and functionality not previously attained in the prior art.

- a. The molecular weight of de-esterified pectin prepared using biocatalytic de-esterification (Example 1) was significantly greater than the molecular weight of de-esterified pectin prepared using conventional de-esterification (Comparative Example 1). Conventional acid de-esterification of pectin resulted in a 34-49% reduction in the molecular weight of the de-esterified pectins. (Paragraph [00261]). Biocatalytic de-esterification of pectin, however, resulted in only a 7-13% reduction in the molecular weight of the de-esterified pectins. (Paragraph [00267]). Thus, biocatalytic de-



esterification of the pectin resulted in significantly less depolymerization of the starting pectin material than conventional de-esterification of pectin.

- b. The intrinsic viscosity of the amidated pectin prepared using biocatalytic de-esterification (Example 1) was significantly greater than the intrinsic viscosity of the amidated pectin prepared using conventional de-esterification (Comparative Example 1). Amidation of the de-esterified pectins prepared using conventional acid de-esterification resulted in a 15-54% reduction in the intrinsic viscosity. (Paragraphs [00279]-[00281]). Amidation of the de-esterified pectins prepared using biocatalytic de-esterification, however, resulted in only a 3-18% reduction in the intrinsic viscosity. (Paragraphs [00284]-[00286]).
- c. Those skilled in the art will appreciate that the intrinsic viscosity of a pectin is directly related to the gel strength of gels made with that pectin. Thus, one skilled in the art would anticipate that synthetic gels having the amidated pectins prepared using conventional de-esterification (Comparative Example 1) would form gels having significantly lower gel strength as compared to synthetic gels having the amidated pectins prepared using biocatalytic de-esterification (Example 1). Rheological measurements of the synthetic gels having representative samples of the amidated pectins from Comparative Example 1 and Example 1 support this understanding. (See e.g., Tables 2.4 and 2.5).

One skilled in the art would not have expected that the amidated pectins prepared using biocatalytic de-esterification would have significantly improved properties as compared to the

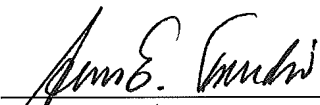


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amidated pectins prepared using conventional acid de-esterification. The significant difference between the properties of the amidated pectins prepared using biocatalytic de-esterification and conventional acid de-esterification is further illustrated in the appended table, and demonstrates that the claimed amidated pectins (1) can clearly be distinguished from the amidated pectins of the prior art, and (2) impart surprising and unexpected improvements to their gels.

10. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

January 20, 2010  
Date

  
Jens E. Trudso



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## APPENDIX

Property	De-esterification Method	Starting Material		
		Lemon	Orange	Lime
DE start %	--	65.7	65.1	64.3
DE end % (de-esterified)	Conventional	33.0	36.6	31.5 40.0
	Bio-catalyst	36.2	39.9	29.8 39.1
DE end % (amidated)	Conventional	9.5	17.7	12.8 14.3
	Bio-catalyst	11.0	20.7	10.9 12.6
DA end % (amidated)	Conventional	21.8	17.0	21.6 13.0
	Bio-catalyst	17.0	14.4	21.7 11.4
MW start (Da)	--	100300	113800	124950
MW end (Da) (de-esterified)	Conventional	65800	60100	73550 66500
	Bio-catalyst	92200	101950	114750 108500
MW (% Reduction) (de-esterified)	Conventional	34.4	47.2	41.1 46.8
	Bio-catalyst	8.1	10.4	8.2 13.2
I.V. (% Reduction) (amidated)	Conventional	27	21	41 40
	Bio-catalyst	15	4	17 13
Gel Strength (Pa) 25°C, 30.8 ppm Ca	Conventional	153	16.9	139 5.6
	Bio-catalyst	545	29.6	526 18
Critical Stress (Pa) 25°C, 30.8 ppm Ca	Conventional	76.04	76.04	48.86 31.4
	Bio-catalyst	286.6	184.2	286.6 76.04
Break Strength (g) 25°C, 30.8 ppm Ca	Conventional	5.50	13.50	5.50 20.20
	Bio-catalyst	46.00	21.53	46.00 32.6